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Analysis of wax esters in edible oils by automated on-line coupling liquid chromatography–gas chromatography using the through oven transfer adsorption desorption (TOTAD) interface

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ABSTRACT

An automated method for the direct analysis of wax esters in edible oils is presented. The proposed method uses the TOTAD (through oven transfer adsorption desorption) interface for the on-line coupling of normal phase liquid chromatography and gas chromatography. In this fully automated system, the oil with C32 wax ester as internal standard and diluted with heptane is injected directly with no sample pre-treatment step other than filtration. The proposed method allows analysis of different wax esters, and is simpler and faster than the European Union Official Method, which is tedious and time-consuming. The obtained results closely match the certified values obtained from the median of the analytical results of the inter-labs certification study. Relative standard deviations of the concentrations are less than 5%. The method is appropriate for routine analysis as it is totally automated.

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1. Introduction

Vegetable oils are mainly composed of triglycerides and complex mixtures of minor compounds of differing nature. These minor compounds can be divided into two groups: those derived from fatty acids, such as wax esters and sterolic esters, and others which are not derived from fatty acids, such as sterols, hydrocarbons and tocopherols.

Analysis of these minor constituents is essential as they are used as a reference for edible oil regulation and for the analytical assessment of oil quality, its origin, the extraction method, the refining procedures used, and possible adulteration of the oils. Determination of the wax ester content has become more important in recent years. The wax esters of analytical interest in vegetable oils are esters of fatty acids with aliphatic alcohols of 40–46 carbon atoms. In olive oils, the analysis of wax esters is used for distinguishing between olive oils of different qualities, such as extra virgin oils and pomace oils (solvent extracted) [1]. Wax esters are mainly located in the epicarp of the olive drupe and, because of their solubility, are more abundant in solvent-extracted oils, while virgin oils contain a lower concentration [2]. The wax ester concentration is used for detecting the admixture of pomace oil to virgin oil. Moreover, low wax ester concentrations are indicative of firm and hard olives, the higher the amount extracted the softer and more degraded the olives are. The European Union has established a legal limit for total wax esters C40–C46 in extra virgin oil (250 mg/kg) and refined olive oil (350 mg/kg) [3].

Besides, the wax esters are useful for determining sunflower oil in safflower oil, two oils that are difficult to distinguish analytically from their other constituents [4]. Therefore, laboratories are faced with the task of performing a greater number of wax ester analyses for quality control purposes.

The most widely used method for the analysis of wax esters is gas chromatography (GC), but prior to the GC analysis wax ester fraction has to be isolated from the triglycerides by means of liquid chromatography (LC) on silica gel [5], thin-layer chromatography (TLC) [6] or by solid phase extraction (SPE) [1,7].

The EU Official Method [3], which involves separating wax esters by silica gel column, the collection of the corresponding fraction and further analysis by GC, is laborious and takes a great amount of time to both prepare the chromatographic silica gel column and elute the sample; the described phases require at least 3 h to be carried out and the operator has to be extremely careful.

Although this method is accurate, the drawbacks mentioned above have led to various attempts being made to reduce the required time and to simplify the analysis procedure. For example, Amelio et al. [2,8] replaced separation on a silica gel column with the more efficient high performance liquid chromatography (HPLC), the wax fraction being collected and then analyzed by GC.

On line coupling LC–GC has the potential for substantial time saving in sample preparation; it eliminates almost all manual work, and allows one person to run a large number of samples in a short

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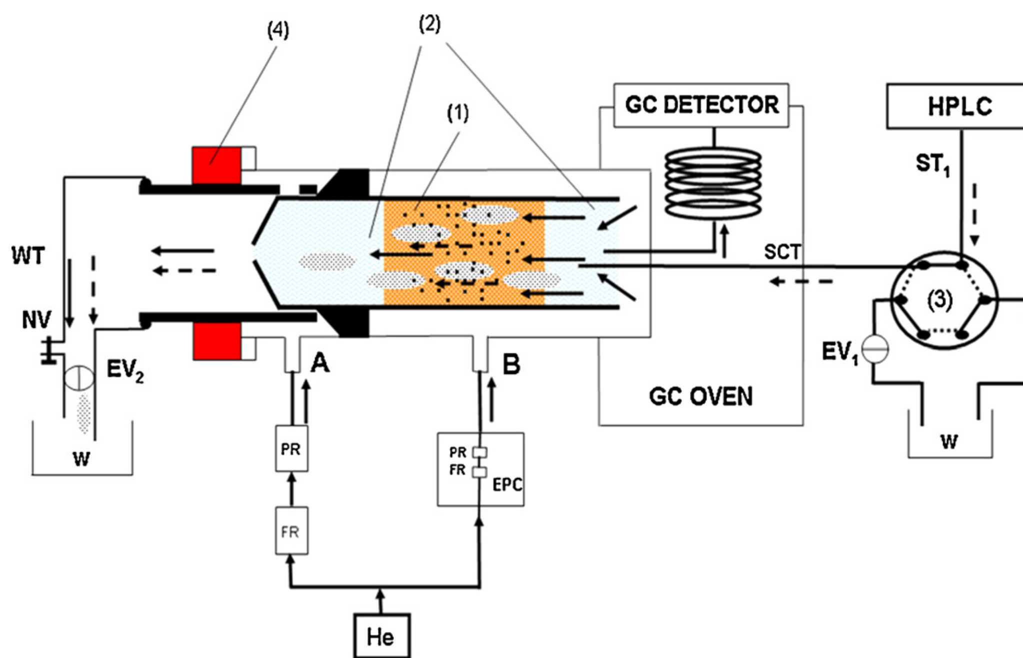

Fig. 1. Automated TOTAD interface during the transfer step. Symbols: (1) sorbent (Tenax TA); (2) glass wool; (3) six-port valve; (4) heated cover; (EV1 and EV2) electrovalves 1 and 2; (EPC) electronic pressure control; (PR) pressure regulator; (FR) flow regulator; (solid arrows) gas flow; (dotted arrows) liquid flow; (ST1) stainless steel tubing, 0.25 mm i.d., to transfer eluent from LC to six-port valve; (SCT) silica capillary tubing, 0.32 mm i.d.; (WT) waste tubing; (W) waste; ( ) solvent; ( ) analytes; NV (⊥) needle valve.

time. On-line coupling LC–GC separates the wax fraction by HPLC and automatically transfers it to the GC to be analyzed [9,10]. On line coupling LC–GC combines the high separation efficiency of HPLC for sample preparation, with the high performance of capillary gas chromatography separation [11].

Grob et al. proposed a LC–GC method that separated the wax ester fraction by normal phase liquid chromatography (NPLC) and transferred the HPLC fraction using concurrent solvent evaporation and a loop-type interface [9,10] or an on-column interface [4]. The low volatility of the wax esters allows use of an on-column interface with concurrent solvent evaporation since solutes are well retained in the retaining precolumn. These authors also analyzed the wax esters by comprehensive two-dimensional GC with flame ionization detection (GC × GC-FID) off-line combined with NPLC–GC, concluding that GC × GC is an excellent tool for understanding the composition of the wax ester fraction but that it is not appropriate for quantitative routine determination.

Nevertheless, the on-line coupling LC–GC by the retention gap techniques using on-column or loop type interfaces with concurrent solvent evaporation has several disadvantages, such as the variability in the retention time in GC and the short life of the chromatographic column, as well as of the capillary used as retention gap.

Our research group has developed an interface named TOTAD (through oven transfer adsorption desorption) [12]. The interface is a modified PTV injector. The changes made in the PTV injector affect the pneumatics, sample introduction and solvent elimination and allows the large volume injection (LVI) of polar solvents [13,14] and the on-line coupling RPLC–GC [15–17]. The TOTAD interface has also been used for LVI of non-polar solvent in GC [18] but, up to date, it has not been used for NPLC–GC coupling. The main problem in on-line coupling LC–GC is to introduce into the GC a volume of eluent proceeding from the LC (the fraction of LC that contains the analytes), which is very superior to that normally injected in GC. Although the introduction of large volume of non-polar solvent into GC using the TOTAD interface has been previously carried out [18], some additional difficulties must be solved in the on line LC–GC. The aim of the present work was to demonstrate the versatility of the TOTAD interface. Since TOTAD interface has been satisfactorily used for LVI of polar and non-polar solvent and for the on-line coupling RPLC–GC, we want to demonstrate the capacity of the system for the on-line coupling LC–GC when LC step is carried out in normal phase. To this aim we have developed a new method to analyze wax esters in edible oils. To analyze wax ester by RPLC–GC is not a good option because wax esters elute after the triglycerides from the LC column when reversed phased is used and due to the tail of the triglyceride's peak, some amount of triglycerides are transferred together the wax ester fractions causing degradations of the GC

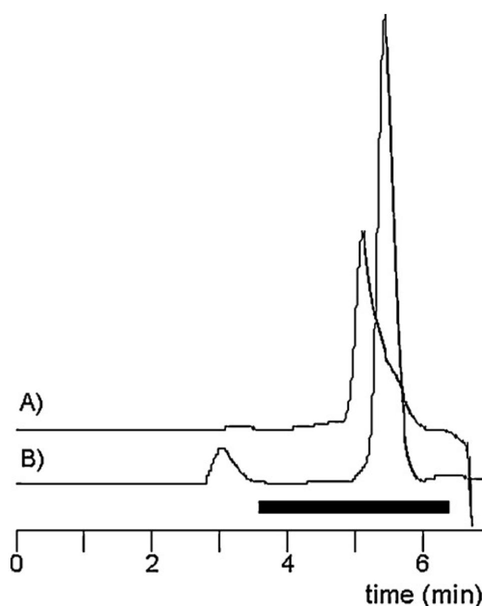

Fig. 2. LC chromatograms of (a) lampante olive oil (sample VG238) and (b) stock solutions of wax esters C32 and C40, registered with RI detector. The thick line between the time axis and the chromatograms indicates the transferred fraction.

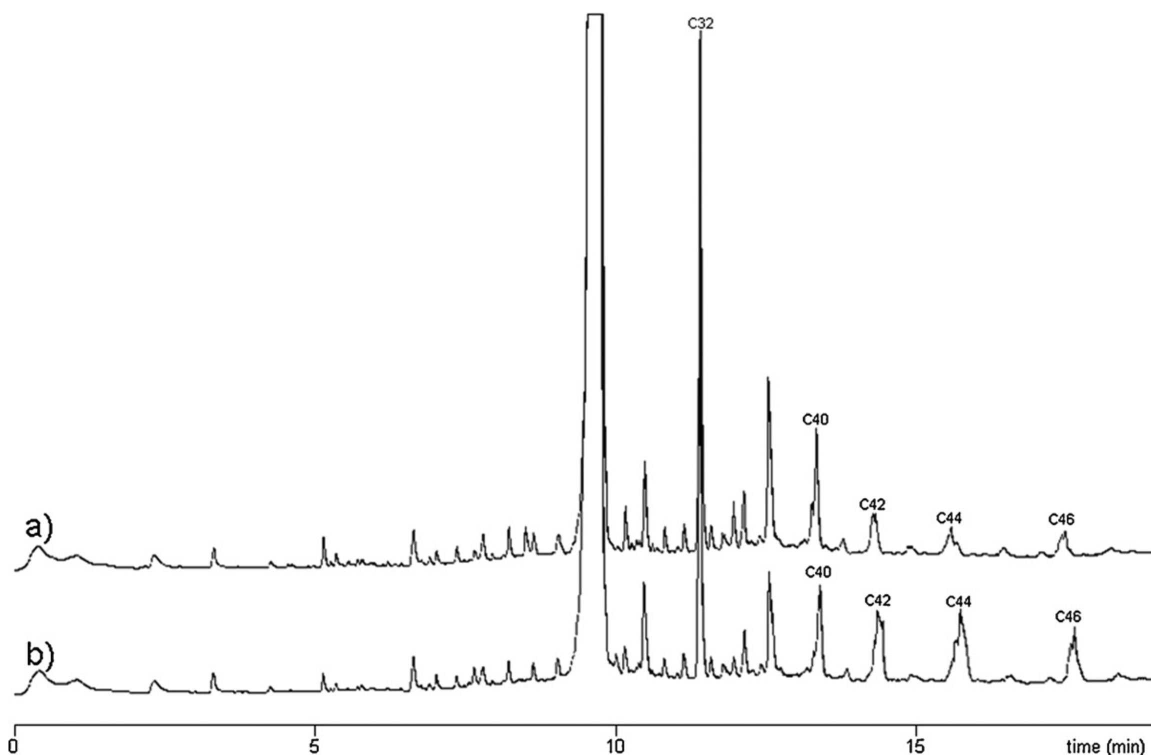

Fig. 3. GC chromatogram obtained from the NPLC–GC-FID analysis of (a) virgin olive oils and (b) refined olive oil.

column, so that to analyze wax esters normal phase should be used in order to avoid this problem.

2. Experimental

2.1. Materials

Different oils were provided by Consejeria de Agricultura y Pesca of Junta de Andalucia, Spain (*VG 238*, lampante olive oil 100%, *BL 597*, a mixture of extra virgin olive oil 60% and sunflower oil 40%, *RF 429*, a mixture of refined olive oil 80% and palm oil 20%, *PM 371* refined pomace olive oil). These oils are certified reference materials which were obtained through the "*Campaña Inter*OLEO-MRC 2006" certification study, organized by the Chemical Metrology and Qualimetrics Unit (CMQ) of the Department of Analytical Chemistry in the University of Granada (Spain). Other oils were purchased from a local market (virgin olive oil, refined olive oil, virgin sunflower oil, refined sunflower oil).

The standards of wax esters, C32 and C40, were purchased from Sigma–Aldrich (Steinheim, Germany), and C36 was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Three stock solutions of 1000 mg/l of each wax ester (C32, C36 and C40) in heptane were prepared and stored a 4 °C. These solutions were used to prepare a standard solution at 3 mg/l by dilution with heptane.

The heptane used to dilute the oil, as well as hexane and ethyl acetate used as mobile phase, all HPLC grade, were purchased from LabScan (Dublin, Ireland).

The glass liner of the TOTAD interface was packed with a 1 cm length of Tenax TA 80–100 mesh (Chrompack, Middelburg, Netherlands) between two plugs of glass wool to keep it in place. The packed liner was conditioned under a helium stream, which was heated from 50 °C to 350 °C at 50 °C/10 min and maintained for 60 min at this final temperature.

Table 1
Relative standard deviation (RSD) for retention time, absolute peak areas, and concentration resulting from the analysis of the wax esters of a lampante olive oil (sample VG 238). The number of injections was 5 ($n = 5$).

Wax ester	RSD (%)		
	Retention time	Area	Concentration
C32	0.08	5.28	–
C40	0.18	5.70	2.80
C42	0.23	6.42	4.84
C44	0.28	4.96	2.36
C46	0.30	4.66	2.11

2.2. NPLC–GC method

2.2.1. Sample preparation

500 mg of the different oil samples were weighed and fortified with 0.5 mg of the internal standard, Lauril Araquidate (C32) (Sigma–Aldrich), when a pomace refined oil was being analyzed and with 0.1 mg in the case of the other oils. The refined pomace oil was diluted with 30 ml of heptane and the other samples with 10 ml, and subsequently filtered through a 0.22 μm filter (Chromatography Research Supplies, Inc.).

2.2.2. Instrumentation

The analyses were performed using on-line coupling LC–GC equipment fitted with an automated TOTAD interface, US Patent 6,402,947 B1 (exclusive rights assigned to KONIK-Tech, Sant Cugat del Vallés, Barcelona, Spain). The TOTAD interface operation mode has been described elsewhere [12].

The HPLC system (Konik model 550) was composed of a quaternary pump, a column oven, a manual injection valve (model 7125 Rheodyne, CA) with a 20 μl loop and an refractive index (RI) detector (Konik RID-560), The gas chromatograph (Konik model HRGC 4000B) was equipped with a TOTAD interface and a FID. KoniKrom

Table 2
Wax ester concentrations (mg/kg) in four different oil samples. The values obtained with the proposed method appear as TOTAD. The concentrations are the average value. The number of injections was 3 ($n = 3$). The certified values obtained according to the official method appear as EU. The difference between the values obtained by both methods appears as percentage (%). For total waxes the expanded uncertainty values appear in italics and between parentheses.[a]

Wax ester	PM 371			VG 238			RF 429			BL 597		
	TOTAD	EU	%	TOTAD	EU	%	TOTAD	EU	%	TOTAD	EU	%
C40	706	656	7.6	55	52.1	5.6	69	68	0.9	42	50	16
C42	1126	1146.3	1.8	94	90.4	4.3	70	68	3.5	41	43,5	5.1
C44	831	810	2.6	105	101	3.8	67	66.9	0.8	18	18	0.6
C46	209	194	8.1	31	31	1.3	31	33	7.0	15	16	9.1
Total	2872(*194*)	2800(*150*)	2.6	285(*17*)	270(*17*)	5.8	237(*2*)	230(*41*)	3.1	116(*5*)	118(*30*)	2.1

[a]The expanded uncertainty was obtained by multiplying the standard deviation by a coverage factor $k = 2$; which defines an interval having a level of confidence of 95%.

Plus 8519 (Konik, Sant Cugat Del Vallés, Barcelona) software was used to obtain data from LC and GC runs and to automate the process.

2.2.3. LC conditions

LC pre-separation was carried out on a 250 mm × 4 mm i.d. column packed with modified silica (Lichrospher 5 μm Si 60, Hichrom, Berks, U.K.) maintained at 25 °C. Hexane/ethyl acetate was used as eluent.

20 μl of the stock solutions were injected in order to ascertain the elution time of the fraction to be transferred to the GC. The initial composition of the eluent (hexane/ethyl acetate; 95:5 (v/v)) at a flow rate of 0.5 ml/min was maintained for 9 min and then the gradient was varied to reach 100% ethyl acetate within 1 min and maintained 20 min to assure the complete elimination of impurities.

For the analysis of the samples, 20 μl of the oil sample prepared as described above was injected. The composition of the eluent, 95:5 (v/v), was maintained constant during elution of the fraction of interest and during the transfer step. After the transfer, the flow was raised to 2 ml/min and the gradient was changed to 100% ethyl acetate within 1 min and maintained for 20 min to ensure complete elimination of triglycerides.

2.2.4. LC–GC transfer

Initially, the TOTAD interface was stabilized at 150 °C with EV1 closed and EV2 open. Helium flow was 500 ml/min through A and through B. The GC oven temperature was maintained at 80 °C. At the beginning, the eluent from the HPLC system was sent to waste. When the front of the wax esters fraction reached the six-port valve, this was automatically switched, transferring the fraction to the GC, this step is outlined in Fig. 1. Helium pushed the solution through the glass liner. During the transfer time, wax esters were retained by the adsorbent inside the glass liner and the solvent was vented to waste through the WT tubing.

When the transfer step was completed, the six-port valve was switched and EV1 opened. In this way, the LC eluent was sent to waste. The solution in the silica capillary tubing SCT was also sent to waste, pushed by the helium. The TOTAD interface temperature was increased from 150 °C to 185 °C and maintained for 2.9 min to ensure elimination of all the remaining solvent and to remove any impurities in the glass-liner. After this time, EV1 and EV2 were closed and flow through B was interrupted while the carrier gas pressure through A was changed to 34 psig.

Then, the TOTAD interface was heated to 350 °C for 5 min to achieve the desorption of the retained solutes and their subsequent transfer to the capillary GC column.

After the GC analysis, EV2 was opened and the interface was cleaned by maintaining the helium stream at 350 °C for 5 min. Finally, it was cooled to 150 °C so that another analysis could be carried out.

2.2.5. GC conditions

A fused-silica column 15 m × 0.25 mm i.d. coated with 5% phenyl methyl silicone (film thickness 0.25 μm) from Quadrex (Weybridge, U.K.) was used for the gas chromatographic separations. During the transfer and the solvent elimination steps, the oven temperature was kept at 80 °C. During GC analysis, the column temperature was maintained at 80 °C for 1 min, raised to 325 °C at 20 °C/min, 5 min, and finally to 335 °C at 20 °C/min, 15 min. The FID temperature was kept at 350 °C. Helium at 34 psig was used as the carrier gas.

2.3. European Union Official Method

The European Union Official Method [3] was applied in the analytical determinations. The certified value is reached from the median of the analytical results of the inter-lab certification studies carried out by fourteen laboratories chosen from among those accredited by the International Olive Oil Council (IOOC) and recommended by the same institution. Six laboratories were from Spain, four from Italy, two from Portugal, one from France and one from Greece.

3. Results and discussion

3.1. Analytical method

The method proposed allows the fully automatic analysis of wax esters in edible oil. The oil is directly injected with no sample pretreatment step other than addition of the internal standard, dilution of the oil sample and filtration. The isolation of the wax ester frac-

Table 3
Wax ester concentrations and standard deviation (mg/kg) obtained by the proposed method for different edible oils (VOO: virgin olive oil; ROO: refined olive oil; VSO: virgin sunflower oil; RSO: refined sunflower oil). The number of injections was 3 ($n = 3$).

Wax ester	VOO		ROO		VSO		RSO	
	Concentration	SD	Concentration	SD	Concentration	SD	Concentration	SD
C40	29.06	0.81	48.09	1.35	16.68	0.47	80.20	2.25
C42	28.49	1.38	72.31	3.50	70.74	3.42	64.21	3.11
C44	20.31	0.48	80.31	1.90	17.05	0.40	35.39	0.84
C46	13.14	0.28	45.24	0.95	n.d.	–	n.d.	–
Total	91.00	2.95	245.95	7.70	104.47	4.29	179.8	6.19

tion from other components of the oils, mainly the triglycerides, is achieved by HPLC in normal phase. The wax ester fraction is automatically transferred to the GC by the TOTAD interface and analyzed with a FID. The complete analysis takes less than 1 h, much shorter than that required when the EU Official Method is used.

3.2. HPLC separation of wax esters

In the proposed method, LC was used for sample preparation, thus avoiding manipulation of the sample and the problems produced in off-line techniques, such as the loss of the analytes and contaminations of the sample. Normal phase was used in HPLC because waxes, which are compounds of long-chain fatty acids with long-chain linear or branched alcohols, are less polar than triglycerides [10], so that they elute before the triglycerides [8]. If reversed phase were used rather than normal phase, the waxes would elute after the triglycerides and it would then be extremely difficult to isolate the wax esters because of the large peak and long triglyceride tail overlapping into the wax ester fraction.

A UV detector would not allow the components of interest to be seen in the LC chromatogram because waxes do not give a good UV response [19]. Therefore, to facilitate detection of the wax ester fraction a RI detector was used.

Before starting, the start and end times of the fraction to be transferred from the LC to GC must be selected in the LC chromatogram. For this, the stock solutions of wax esters (1000 mg/l) were injected. It is especially important to establish the experimental conditions in such a way that the wax ester fraction does not overlap the major components of the oil, especially the triglycerides, in the LC pre-separation step [10,20]. Fig. 2a shows the LC chromatogram of a lampante olive oil (sample VG 238), while Fig. 2b corresponds to the LC analysis of a standard mixture of C32 and C40 obtained using an RI detector.

The wax ester fraction transferred to the GC, indicated in the Fig. 2, does not overlap the triglyceride fraction. Wax esters elute between 3.50 and 6.16 min. The flow rate in the LC system was 0.5 ml/min, so that the resulting volume of the fraction was 1.33 ml, which is the volume that must be transferred from the LC to the GC. After the complete elution of the wax esters from the HPLC column, the triglycerides were eluted by increasing the flow and changing the eluent composition to 100% ethyl acetate.

3.3. Transfer and GC analysis

The volume of the fraction to be transferred is not a problem because the TOTAD interface eliminates the eluent totally as evaporative and non-evaporative mode. However, in this case it makes no sense to transfer a volume larger than 1.33 ml. Bearing in mind that the flow during the transfer step is 0.5 ml/min, it takes 2.66 min to complete this step.

Wax esters have a very high molecular weight and extremely low volatility. Therefore, these compounds are well retained in the adsorbent inside the liner, while the eluent is easily eliminated, even maintaining an interface temperature as high as 150 °C during the transfer step.

Fig. 3 shows the GC chromatogram resulting from the analysis of a virgin olive oil and a refined olive oil. The chromatogram is very clear considering that a FID was used. The peaks of the wax esters, characterized by the total carbon number, are easily identified according to their retention time, related to the retention time of C32 and C40 as in the EU Official Method [3]. Quantitative determinations were obtained based on the internal standard (wax ester C32).

Due to the highly complex composition of edible oils, other compounds eluting in LC along side the waxes can be seen in the GC chromatogram. Nevertheless, these compounds, which have not been identified, did not interfere in the analysis of the wax esters.

3.4. Repeatability of the method

The precision obtained with the NPLC–GC method was evaluated. Table 1 shows the relative standard deviation (RSDs) of the retention times, absolute peak areas, and concentration of wax esters obtained from five injections of a lampante olive oil (sample VG 238).

No variability in retention times was observed (RSDs varied from 0.08 to 0.30). For the absolute peak areas, the RSDs were less than 6.5%. The RSDs for the concentration calculated from the internal standard were less than 5% in all cases. Bearing in mind that these values correspond to the full analysis, it confirm that on-line LC–GC-FID is a highly repeatable method. Moreover, the low RSDs of the absolute peak areas open up the possibility of a further simplification of the method by avoiding the use of an internal standard.

3.5. Analysis of edible oil samples

The proposed method was applied to the analysis of four different oil samples which had also been analyzed by the EU Official Method. Table 2 shows the data obtained with both methods and the difference (%) between the values. The values show good agreement for the individual wax esters and for their total contents (sum of wax esters from C40 to C46). The difference between the individual wax esters is less than 10%, ranging from 0.6 to 9.1%, except for C40 in sample BL597 which is 16%. The data of the certified values for the individual wax esters are not given with their corresponding uncertainty and this is given only in the case of the value of the total wax esters. As can be observed in Table 2, for total wax esters, the difference between the certified values and the values obtained by the NPLC–GC method using the TOTAD interface is less than 6%. The uncertainty obtained with the latter is lower that that given in the certified values, except for sample PM371.

After optimizing the method and verifying that the data obtained were similar to those obtained with the official method, four commercial edible oils were analyzed.

Table 3 presents the concentration of the wax esters (C40–C46) for four different edible oils. As expected, the concentrations of total wax esters were higher in refined oils than in virgin oils (olive and sunflower). Some authors [4] have pointed to a correlation between chemical analysis results and sensorial evaluation, extra virgin olive oils with a low content of straight chain wax esters being evaluated as high quality oils, while some oils with a high content were thought not to be of extra virgin quality.

4. Conclusions

In the present work the suitability of the TOTAD interface for the on line coupling NPLC–GC has been demonstrated and a new method to analyze wax esters in edible oils by NPLC–GC using the TOTAD interface has been developed. The proposed method is rapid and straightforward compared to the EU Official Method since the number of operations needed is reduced and it is practically totally automatic. The wax ester contents obtained with the described procedure were very close to those obtained by the EU Official Method. Repeatability was also valid as RSDs for concentrations are less then 5%. The new method could be considered as very useful for routine analysis purposes.

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References

[1] G. Nota, D. Naviglio, R. Romano, V. Sabia, S.S. Musso, C. Improta, J. Agric. Food Chem. 47 (1999) 202.
[2] M. Amelio, R. Rizzo, F. Varazini, J. Am. Oil Chem. Soc. 75 (1998) 527.
[3] EC 2007/702, Off. J. Eur. Union L161 (2007) 11.
[4] M. Biedermann, P. Haase-Aschoff, K. Grob, Eur. J. Lipid Sci. Technol. 110 (2008) 1084.
[5] C. Mariani, E. Fedeli, Riv. Ital. Sost. Grasse 66 (1989) 397.
[6] K. Kawanishi, K. Aoki, Y. Hashimoto, A. Matsunobu, J. Am. Oil Chem. Soc. 68 (1991) 869.
[7] B. Reiter, M. Lechner, E. Lorbeer, R. Aichholz, J. High Res. Chromatogr. 22 (1999) 514.
[8] M. Amelio, R. Rizzo, F. Varazini, J. Am. Oil Chem. Soc. 70 (1993) 793.
[9] K. Grob, T. Läubli, J. High Res. Chromatogr. 9 (1986) 593.
[10] K. Grob, M. Lanfranchi, C. Mariani, J. High Res. Chromatogr. 471 (1989) 397.
[11] T. Hyötyläinen, M.L. Riekkola, J. Chromatogr. A 1000 (2002) 357.
[12] M. Pérez, J. Alario, A. Vázquez, J. Villén, J. Microcol. Sep. 11 (1999) 582.
[13] J. Alario, M. Pérez, A. Vázquez, J. Villén, J. Chromatogr. Sci. 39 (2001) 65.
[14] R.M. Toledano, J.M. Cortés, J.C. Andini, J. Villén, A. Vázquez, J. Chromatogr. A 1217 (2010) 4738.
[15] R. Sánchez, D. Riquelme, A. Vázquez, J. Villén, J. Agric. Food Chem. 51 (2003) 6098.
[16] R. Sánchez, A. Vázquez, J.C. Andini, J. Villén, J. Chromatogr. A 1029 (2004) 167.
[17] J.M. Cortés, R. Sánchez, J. Villén, A. Vázquez, J. Agric. Food Chem. 54 (2006) 6963.
[18] J.M. Cortés, R. Sánchez, E.M. Díaz-Plaza, J. Villén, A. Vázquez, J. Agric. Food Chem. 54 (2006) 1997.
[19] K. Taek Hwang, S.L. Cuppett, C.L. Weller, M.A. Hanna, J. Sep. Sci. 25 (2002) 619.
[20] M. Biedermann, K. Grob, W. Meier, J. High Res. Chromatogr. 12 (1989) 591.